INHIBITORY ACTION OF HETEROLOGOUS PROTEIN MIXTURES ON ANAPHYLAXIS *

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In studying the absorption of serum from the subcutaneous tissues, a delicate method was needed to detect small amounts of foreign protein in the circulation. Since from many investigations (Rosenau and Anderson,[1] and Wells,[2]) we know that extremely small amounts of protein will sensitize guinea-pigs, the anaphylaxis reaction was indicated as an excellent means to measure the amount, rate, and channel of absorption of serum from the subcutaneous tissues. This method has been used by several investigators to show the presence of foreign protein in the circulation, most recently by Eleanor Van Ness Van Alstyne,[3] who asserts that she has thus shown whole protein to be absorbed through the gastro-intestinal tract.

Our method of procedure was to anesthetize a medium-sized dog and insert cannulae into the thoracic duct and a small artery in the neck. Varying amounts of horse serum were injected under the skin of the abdomen, and at stated intervals of time samples of 5 to 10 c.c. lymph and blood were taken. From each sample 1.5 to 2 c.c. of serum were injected intraperitoneally into guinea-pigs. After fourteen days, these were tested for sensitization by injecting intraperitoneally 0.5 to 1 c.c. horse serum.

Negative results were always obtained, even under conditions where we had reason to believe that large enough amounts of horse serum to sensitize guinea-pigs ought to have been contained in 2 c.c. of serum. Under similar conditions, colloid dyes of large, molecular dimension appeared promptly in the blood and lymph. In an attempt to obtain positive results, 50 c.c. of horse serum were forced under the skin of a dog with compressed air, in much the same way that anatomists inject the subcutaneous lymphatics with mercury; but even after this drastic procedure, the dog's blood did not sensitize guinea-pigs

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1. Hygienic Laboratory Bull. 29, 1906.
2. Jour. Infect. Dis., 1911, 9, p. 449.
3. Arch. Int. Med., 1913, 12. p. 372.

to horse serum. Sensitized guinea-pigs, when injected subcutaneously with large amounts of antigen in this way, die promptly as with an intravenous injection.

In order to find wherein the difficulty lay, the following experiment was made to find whether a mixture of dog serum with a definite amount of horse serum could sensitive guinea-pigs to horse serum.

On October 7, 1914, a dog under ether was bled aseptically from the carotid artery. After coagulation, the blood was centrifugated and the clear serum drawn off. Into each of six test tubes were immediately put 2 c.c. of the serum. To these test tubes were added respectively 0.1, 0.01, 0.001, 0.0001, and 0.00001 c.c. horse serum. Each amount of horse serum was diluted with NaCl solution so that it was contained in 1 c.c. The tubes were put in the ice-box over night. The next morning they were heated to 56 C. for one-half hour. Fresh dog serum is toxic, and to avoid killing the guinea-pigs, it is usually either heated or injected at intervals. Twenty-four hours after the blood was withdrawn, the contents of each tube were injected intraperitoneally into a guinea-pig. As controls, two guinea-pigs were given 0.1 and 0.01 c.c. horse serum diluted to 3 c.c.

On October 26, all guinea-pigs received intraperitoneally, 1 c.c. horse serum diluted to 2 c.c. The results are seen in Table 1.

TABLE 1

THE EFFECT OF DOG SERUM ON THE ACTIVE SENSITIZATION OF GUINEA-PIGS WITH HORSE SERUM

Guinea-Pigs	First Injection	Reaction After Second Injection (Intraperitoneal) of 1 c.c. Horse Serum Diluted One-Half
1	0.1 c.c. horse serum 2 c.c. dog serum	Slight ruffling of coat
2	0.01 c.c. horse serum 2 c.c. dog serum	Questionable
3	0.001 c.c. horse serum 2 c.c. dog serum	None
4	0.0001 c.c. horse serum 2 c.c. dog serum	None
5	0.00001 c.c. horse serum 2 c.c. dog serum	None
6	0.1 c.c. horse serum 2 c.c. NaCl solution	Dead in 35 min.
7	0.01 c.c. horse serum 2 c.c. NaCl solution	Marked within 10 min.; prostrated in 25 min. Symptoms lasted over 6 hours. Recovered

The results show that anaphylactic sensitization has been almost completely suppressed as the result of mixing the sensitizing dose with dog serum. To make sure, the experiment was repeated several times, using more complete controls.

On November 2, 1.5 c.c. were measured from nine dilutions of horse serum that contained in each cubic centimeter, respectively, 0.5, 0.1, 0.01, 0.002, 0.001, 0.0001, 0.00005, 0.00002 and 0.00001 c.c. of horse serum. This amount was put into test tubes containing 3 c.c. of freshly drawn dog serum. Similar amounts were measured into test tubes containing 3 c.c. of NaCl solution. All tubes were well shaken and kept at room temperature twenty-one hours, then heated to 56 C. for one-half hour. When cooled to body temperature, 3 c.c. from each tube were injected intraperitoneally into a guinea-pig. Thus each animal received 2 c.c. of dog serum or NaCl solution plus the amount of horse serum contained in 1 c.c. of one of the dilutions.

On November 10, each animal received 1 c.c. of horse serum diluted to 2 c.c. The results are seen in Table 2.

TABLE 2

THE EFFECT OF DOG SERUM ON THE ACTIVE SENSITIZATION OF GUINEA-PIGS WITH HORSE SERUM

Guinea-Pigs	First Injection	Reaction After Second Injection (Intraperitoneal) of 1 c.c. Horse Serum Diluted One-Half
1	0.1 c.c. horse serum 2 c.c. dog serum	None
2	0.01 c.c. horse serum 2 c.c. dog serum	Scratches nose occasionally
3	0.002 c.c. horse serum 2 c.c. dog serum	None
4	0.001 c.c. horse serum 2 c.c. dog serum	None
5	0.0001 c.c. horse serum 2 c.c. dog serum	None
6	0.00005 c.c. horse serum 2 c.c. dog serum	None
7	0.00002 c.c. horse serum 2 c.c. dog serum	None
8	0.00001 c.c. horse serum 2 c.c. dog serum	None
9	0.01 c.c. horse serum 2 c.c. NaCl solution	Marked; prostrated. Died during night
10	0.002 c.c. horse serum 2 c.c. NaCl solution	Marked; prostrated. Recovered
11	0.001 c.c. horse serum 2 c.c. NaCl solution	Died during night
12	0.0001 c.c. horse serum 2 c.c. NaCl solution	Severe; prostrated. Recovered
13	0.00005 c.c. horse serum 2 c.c. NaCl solution	Strongly positive
14	0.00002 c.c. horse serum 2 c.c. NaCl solution	Positive
15	0.00001 c.c. horse serum 2 c.c. NaCl solution	Positive

On November 23, 9 c.c. of the serum from freshly drawn dog blood were mixed thoroughly with 1 c.c. of horse serum. The mixtures were put into the incubator at 38 C. for two hours. A few drops of $CHCl_3$ were then added and the whole kept at room temperature until the next morning. The following dilutions of the mixture were then made: 1:10, 1:50, 1:100, 1:1000, 1:10000, 1:20000. One cubic centimeter of these dilutions contained therefore, 0.01, 0.002, 0.001, 0.0001, 0.00005, 0.00002, and 0.00001 c.c. of horse serum and nine times as much dog serum. A similar series was made using NaCl solution instead of dog serum. One cubic centimeter of each of the dilutions was injected intraperitoneally into a guinea-pig.

TABLE 3

THE EFFECT OF DOG SERUM ON THE ACTIVE SENSITIZATION OF GUINEA-PIGS WITH HORSE SERUM

Guinea-Pigs	First Injection	Reaction After Second Injection (Intraperitoneal) of 1 c.c. Horse Serum Diluted One-Half
1	0.01 c.c. horse serum in dog serum	None
2	0.002 c.c. horse serum in dog serum	None
3	0.001 c.c. horse serum in dog serum	None
4	0.0001 c.c. horse serum in dog serum	None
5	0.00005 c.c. horse serum in dog serum	None
6	0.00002 c.c. horse serum in dog serum	None
7	0.00001 c.c. horse serum in dog serum	None
8	0.01 c.c. horse serum in NaCl solution	Very severe; prostrated in 1 hour Recovered
9	0.002 c.c. horse serum in NaCl solution	Very severe; prostrated in 1 hour Recovered
10	0.001 c.c. horse serum in NaCl solution	Very severe; prostrated. Recovered
11	0.0001 c.c. horse serum in NaCl solution	Marked. Recovered
12	0.00005 c.c. horse serum in NaCl solution	None
13	0.00002 c.c. horse serum in NaCl solution	None
14	0.00001 c.c. horse serum in NaCl solution	None

On December 3, all animals received intraperitoneally 1 c.c. of horse serum. The results are shown in Table 3.

On December 21, to 9 c.c. of fresh dog serum and to 9 c.c. of NaCl solution, 1 c.c. of horse serum was added and well mixed. The mixtures were put

into the incubator two hours, and into the ice-box over night (twenty hours). Dilutions of each of the mixtures were made so that in 5 c.c. would be contained 0.5 c.c., 0.25 c.c., 0.1 c.c., 0.02 c.c., 0.01 c.c., and 0.002 c.c. horse serum. Five cubic centimeters of each of these dilutions were injected into a guinea-pig The dilutions that contained 0.5 c.c. horse serum were given in three injections at intervals of one-half hour (in order to prevent the toxic action of fresh dog serum) and those that contained 0.25 c.c. were given in two injections at an interval of one-half hour. The other dilutions were given in one injection.

On January 14, all animals were given, intraperitoneally, 1 c.c. horse serum diluted to 2 c.c. The results are seen in Table 4.

TABLE 4

THE EFFECT OF DOG SERUM ON THE ACTIVE SENSITIZATION OF GUINEA-PIGS WITH HORSE SERUM

Guinea-Pigs	First Injection	Reaction After Second Injection (Intraperitoneal) of 1 c.c. Horse Serum Diluted One-Half
1	0.5 c.c. horse serum in dog serum	Very slight
2	0.25 c.c. horse serum in dog serum	Slight restlessness
3	0.1 c.c. horse serum in dog serum	Restless
4	0.02 c.c. horse serum in dog serum	None
5	0.01 c.c. horse serum in dog serum	None
6	0.002 c.c. horse serum in dog serum	None
7	0.5 c.c. horse serum in NaCl solution	Immediate; convulsions; died during night
8	0.25 c.c. horse serum in NaCl solution	Severe. Recovery
9	0.1 c.c. horse serum in NaCl solution	Severe. Recovery
10	0.02 c.c. horse serum in NaCl solution	Dead in 30 minutes
11	0.01 c.c. horse serum in NaCl solution	Very severe; prostrated. Recovered
12	0.002 c.c. horse serum in NaCl solution	Very severe. Recovery

It will be seen that the results are consistent. While the controls showed the usual symptoms of anaphylaxis, the guinea-pigs that received dog serum showed few or no symptoms. The difference was shown very strikingly when, after the toxogenic dose, the cage containing one series was placed beside the one containing the other series.

In seeking for an explanation of this phenomenon, it was at first thought that the dog serum had a specific action on the horse serum of an antibody-antigen nature which attenuated its ability to act as an antigen, because in one instance there was a slight precipitate seen in three of the dog-serum horse-serum mixtures (Table 3, Guinea-pigs 1, 2, and 3). In order to find whether this property was confined to

TABLE 5

THE EFFECT OF DOG SERUM AND EGG-WHITE ON THE ACTIVE SENSITIZATION OF GUINEA-PIGS WITH HORSE SERUM

Guinea-Pigs	First Injection	Second Injection (Intraperitoneal)	Reaction
1	1/3 c.c. horse serum 2 c.c. dog serum	0.5 c.c. horse serum diluted one-half	Indefinite
2	1/6 c.c. horse serum 2 c.c. dog serum	0.5 c.c. horse serum diluted one-half	Slight
3	1/15 c.c. horse serum 2 c.c. dog serum	0.5 c.c. horse serum diluted one-half	None
4	1/75 c.c. horse serum 2 c.c. dog serum	1 c.c. horse serum diluted one-half	None
5	1/150 c.c. horse serum 2 c.c. dog serum	1 c.c. horse serum diluted one-half	None
6	1/750 c.c. horse serum 2 c.c. dog serum	1 c.c. horse serum diluted one-half	None
7	1/3 c.c. horse serum 2 c.c. egg-white solution	0.5 c.c. horse serum diluted one-half	None
8	1/6 c.c. horse serum 2 c.c. egg-white solution	0.5 c.c. horse serum diluted one-half	None
9	1/15 c.c. horse serum 2 c.c. egg-white solution	0.5 c.c. horse serum diluted one-half	None
10	1/75 c.c. horse serum 2 c.c. egg-white solution	1 c.c. horse serum diluted one-half	None
11	1/150 c.c. horse serum 2 c.c. egg-white solution	1 c.c. horse serum diluted one-half	None
12	1/750 c.c. horse serum 2 c.c. egg-white solution	1 c.c. horse serum diluted one-half	None
13	1/3 c.c. horse serum 2 c.c. NaCl solution	0.5 c.c. horse serum diluted one-half	Very severe; prostrated. Recovery.
14	1/6 c.c. horse serum 2 c.c. NaCl solution	0.5 c.c. horse serum diluted one-half	Severe; prostrated. Recovery
15	1/15 c.c. horse serum 2 c.c. NaCl solution	0.5 c.c. horse serum diluted one-half	Very marked
16	1/75 c.c. horse serum 2 c.c. NaCl solution	1 c.c. horse serum diluted one-half	Dead in 25 minutes
17	1/150 c.c. horse serum 2 c.c. NaCl solution	1 c.c. horse serum diluted one-half	Dead in 60 minutes
18	1/750 c.c. horse serum 2 c.c. NaCl solution	1 c.c. horse serum diluted one-half	Very marked

dog serum, the experiment was repeated using human serum and cat serum. Results followed identical in nature with those obtained with dog serum.

As the sera of man, dog, and cat are very toxic for guinea-pigs, another possibility was conceived, namely, that they acted deleteriously

TABLE 6

SENSITIZATION OF GUINEA-PIGS WITH A LARGE AMOUNT OF AN ANTIGEN INJECTED WITH A SMALLER AMOUNT OF ANOTHER

Guinea-Pigs	Previous Treatment	Re-injection (Intraperitoneal)	Reaction
1	11/2/14 0.1 c.c. horse serum 2 c.c. dog serum 11/10/14 1 c.c. horse serum (See Table 2, No. 1)	11/12/14 1 c.c. inactivated dog serum	Slight
2	11/2/14 0.1 c.c. horse serum 2 c.c. dog serum 11/2/14 1 c.c. horse serum (Table 2, No. 2)	11/21/14 1 c.c. inactivated dog serum	Very severe. Recovered
3	2/12/15 0.02 c.c. horse serum 2 c.c. human serum 3/1/15 1 c.c. horse serum (See Table 4, No. 4)	3/18/15 1 c.c. inactivated human serum	Death in 45 minutes
4	1/28/15 $\frac{1}{3}$ c.c. horse serum 2 c.c. dog serum 2/13/15 1 c.c. horse serum (See Table 5, No. 1)	2/16/15 1 c.c. inactivated dog serum	Slight
5	1/28/15 $\frac{1}{75}$ c.c. horse serum 2 c.c. of 50% egg-white solution 2/13/15 1 c.c. horse serum (See Table 5, No. 10)	2/29/15 1 c.c. of 50% egg-white solution	Very marked; prostration. Recovery
6	3/31/15 0.002 gm. egg albumin 2 c.c. dog serum	4/16/15 1 c.c. inactivated dog serum	Marked
7	3/31/15 0.0005 gm. egg albumin 2 c.c. dog serum	4/30/15 1 c.c. inactivated dog serum	Dead in 35 minutes

on the body cells of the guinea-pig so that they were unable to produce antibodies. To see whether toxicity was a factor, a non-toxic protein, egg-white, was used to replace the sera. Egg-white diluted one-half contains about the same amount of protein as dog serum. The experiment was conducted as follows:

On January 28, dilutions of horse serum were made so that 1 c.c. contained respectively 1/3 c.c., 1/6 c.c., 1/15 c.c., 1/75 c.c., 1/150 c.c., and 1/750 c.c. One cubic centimeter of each dilution was put into each of three test tubes so that there were three identical series of six test tubes each. To the tubes of one series were added 3 c.c. of fresh dog serum; to those of another, were added 3 c.c. of a 50 percent solution of whole egg-white. The mixtures were put into the incubator for two hours and into the ice-box for twelve hours. Three cubic centimeters from each tube were injected into guinea-pigs. The animals receiving 1/15 c.c. horse serum + 2 c.c. dog serum, and 1/750 c.c. horse serum + 2 c.c. dog serum, died during the night following the injection.

TABLE 7

THE INHIBITORY ACTION OF EGG-WHITE WHEN INJECTED PREVIOUSLY TO A SENSITIZING DOSE OF HORSE SERUM

Guinea-Pigs	Preliminary Treatment	Toxogenic Dose	Reaction
1	3/24/15 2 c.c. of a 5% solution egg albumin 3/25/15 2 c.c. of a 5% solution egg albumin 3/26/15 0.1 c.c. horse serum	2 c.c. horse serum (intraperitoneal) 4/13/15	Very slight
2	3/24/15 2 c.c. of a 5% solution egg albumin 3/25/15 2 c.c. of a 5% solution egg albumin 3/26/15 0.1 c.c. horse serum	2 c.c. horse serum (intraperitoneal) 4/13/15	Questionable
3	3/24/15 2 c.c. of a 5% solution egg albumin 3/25/15 2 c.c. of a 5% solution egg albumin 3/26/15 0.01 c.c. horse serum	2 c.c. horse serum (intraperitoneal) 4/13/15	None
4	3/24/15 2 c.c. of a 5% solution egg albumin 3/25/15 2 c.c. of a 5% solution egg albumin 3/25/15 0.001 c.c. horse serum	1 c.c. horse serum (intracardiac) 4/13/15	None
5	0.1 c.c. horse serum	2 c.c. horse serum (intraperitoneal) 4/13/15	Very severe
6	0.1 c.c. horse serum	2 c.c. horse serum (intraperitoneal) 4/13/15	Very severe
7	0.01 c.c. horse serum	2 c.c. horse serum (intraperitoneal) 4/13/15	Very severe
8	0.001 c.c. horse serum	1 c.c. horse serum (intracardiac) 4/13/15	Dead in 3 minutes

On February 13, all animals received intraperitoneally, horse serum diluted one-half. The results are seen in Table 5.

Egg-white, then, is just as efficient in inhibiting anaphylactic sensitization to horse serum as dog, cat, or human sera. Altho sensitization to horse serum is inhibited, there is sensitization to the inhibiting protein which is more marked after four weeks than after two weeks because of its large amount (see Table 6).

Egg-white will produce this inhibition, not only when mixed with the horse serum, but also when injected into the guinea-pigs before the horse serum is injected (described in Table 7).

In order to see whether there is any inhibition of the toxigenic action of an antigen by another protein, the following experiments were made:

On April 2, eight guinea-pigs received intraperitoneally 0.001 gm. Merck's egg albumin contained in 1 c.c. NaCl solution.

On April 15, the animals received intravenously varying amounts of egg albumin contained in 1 c.c. NaCl solution + 2 c.c. horse serum or 2 c.c. NaCl solution, with results as shown in Table 8.

TABLE 8

EFFECT OF HORSE SERUM ON THE TOXIGENIC ACTION OF EGG ALBUMIN

Guinea-Pigs	First Injection (Intraperitoneal)	Second Injection (Intravenous)	Reaction
1	0.001 gm. egg albumin	0.1 gm. egg albumin	Death immediately
2	0.001 gm. egg albumin	0.01 gm. egg albumin	Death in 5 minutes
3	0.001 gm. egg albumin	0.0001 gm. egg albumin	Death in 7.5 minutes
4	0.001 gm. egg albumin	0.0001 gm. egg albumin 2 c.c. horse serum	Death in 6.75 minutes
5	0.001 gm. egg albumin	0.0001 gm. egg albumin	Death in 7 minutes
6	0.001 gm. egg albumin	0.0001 gm. egg albumin 2 c.c. horse serum	Death in 5 minutes
7	0.001 gm. egg albumin	0.000001 gm. egg albumin	Marked. Recovery
8	0.001 gm. egg albumin	0.000001 gm. egg albumin 2 c.c. horse serum	Marked. Recovery

On April 19, eight guinea-pigs were given, intraperitoneally, 0.1 c.c. horse serum.

Later in April, the guinea-pigs were given varying amounts of horse serum + 2 c.c. of a 10 percent solution of Merck's egg albumin or 2 c.c. of NaCl solution mixed just before the injection. The results are seen in Table 9.

Recently Dr. H. J. Corper, in a personal communication, described experiments which were similar to those of Van Alstyne, made by

Dr. J. H. McClellan and himself in the physiological laboratories of the University of Illinois. Large quantities of egg-white were fed to dogs which were bled at regular intervals. The serum of these animals, even after the largest quantity of egg-white had been given, never sensitized guinea-pigs to egg-white. For this reason, the work was temporarily discontinued. At his request the next experiment was made.

TABLE 9

THE EFFECT OF EGG ALBUMIN ON THE TOXOGENIC ACTION OF HORSE SERUM

Guinea-Pigs	First Injection (Intraperitoneal)	Second Injection (Intravenous)	Reaction
1	1 c.c. horse serum	0.5 c.c. horse serum 2 c.c. NaCl	Dead in 10 minutes
2	1 c.c. horse serum	0.5 c.c. horse serum 0.2 gm. egg albumin	Dead in 8 minutes
3	1 c.c. horse serum	0.1 c.c. horse serum 2 c.c. NaCl	Dead in 35 minutes
4	1 c.c. horse serum	0.1 c.c. horse serum 0.2 gm. egg albumin	Dead in 43 minutes
5	1 c.c. horse serum	0.01 c.c. horse serum 2 c.c. NaCl	Very severe. Recovery
6	1 c.c. horse serum	0.01 c.c. horse serum 0.2 gm. egg albumin	Very severe. Recovery

On March 31, dilutions of egg albumin, crystallized by the Hopkins-Cole method, were made so that 1 c.c. contained respectively 0.005, 0.003, 0.001, and 0.0001 gm. of egg albumin. One cubic centimeter from each of these dilutions was mixed with 2 c.c. of fresh dog serum and another was mixed with 2 c.c. NaCl solution and injected into guinea-pigs intraperitoneally.

On April 16, each of the animals received 0.2 gm. egg albumin in 4 c.c. NaCl solution, intraperitoneally. The results are given in Table 10.

The results of this experiment are the most striking of all. All the control animals died, while those receiving dog serum with the sensitizing dose showed slight, if any, reaction.

In regard to another hypothesis that will be discussed farther on, an experiment was made to find the effect of egg-white on the passive sensitization of guinea-pigs. A rabbit was sensitized by repeated intraperitoneal injections of horse serum. At the end of two weeks, the rabbit was bled from the heart. Two series of guinea-pigs were each injected intraperitoneally with 2 c.c., 1 c.c. 0.3 c.c., and 0.1 c.c. of this serum. In one series, the serum was mixed with 2 c.c. of a 10 percent solution of Merck's egg albumin just before being injected

TABLE 10

THE INHIBITORY ACTION OF DOG SERUM ON ACTIVE SENSITIZATION WITH EGG ALBUMIN

Guinea-Pigs	First Injection	Reaction After Second Injection (Intraperitoneal) of 0.2 gm. Egg Albumin
1	0.005 gm. egg albumin 2 c.c. dog serum	Slight
2	0.003 gm. egg albumin 2 c.c. dog serum	Questionable
3	0.001 gm. egg albumin 2 c.c. dog serum	None
4	0.0001 gm. egg albumin 2 c.c. dog serum	None
5	0.005 gm. egg albumin 2 c.c. NaCl	Dead in 55 minutes
6	0.003 gm. egg albumin 2 c.c. NaCl	Dead in 28 minutes
7	0.001 gm. egg albumin 2 c.c. NaCl	Dead in 15 minutes
8	0.0001 gm. egg albumin 2 c.c. NaCl	Dead in 34 minutes

TABLE 11

THE EFFECT OF EGG PROTEIN ON PASSIVE SENSITIZATION

Guinea-Pigs	First Injection	Second Injection	Reaction
1	2 c.c. immune rabbit serum + 0.2 gm. egg albumin	0.2 c.c. horse serum (intravenous)	Very slight
2	2 c.c. immune rabbit serum	0.2 c.c. horse serum (intravenous)	Dead in 5 minutes
3	1 c.c. immune rabbit serum + 0.2 gm. egg albumin	0.2 c.c. horse serum (intravenous)	Slight
4	1 c.c. immune rabbit serum	0.2 c.c. horse serum (intravenous)	Very severe; died in 4 hours
5	0.3 c.c. immune rabbit serum + 0.2 gm. egg albumin	0.2 c.c. horse serum (intracardiac)	Slight
6	0.3 c.c. immune rabbit serum	0.2 c.c. horse serum (intracardiac)	Marked
7	0.1 c.c. immune rabbit serum + 2 gm. egg albumin	0.2 c.c. horse serum (intracardiac)	None
8	0.1 c.c. immune rabbit serum	0.2 c.c. horse serum (intracardiac)	Very slight

into the guinea-pigs. At the end of twenty-four hours each animal received 0.2 c.c. horse serum intravenously. As Table 11 shows, there was a marked inhibition of passive sensitization by means of the egg albumin. Also, as with active sensitization, passive sensitization was inhibited when the inhibiting protein was injected into the animal before the sensitizing protein (Table 12).

TABLE 12

THE INHIBITORY ACTION OF EGG ALBUMIN ON PASSIVE SENSITIZATION WHEN INJECTED BEFORE THE SENSITIZING SERUM

Guinea-Pigs	First Injection	Second Injection	Reaction After Third Injection (Intravenous), 24 Hours After the First, of 0.2 c.c. Horse Serum
1	0.2 gm. egg albumin	1 c.c. horse immune rabbit serum 24 hours after first injection	None
2	0.2 gm. egg albumin	1 c.c. horse immune rabbit serum 6 hours after first injection	Questionable
3	0.2 gm. egg albumin	1 c.c. horse immune rabbit serum 4 hours after first injection	None
4	0.2 gm. egg albumin	1 c.c. horse immune rabbit serum 2 hours after first injection	Very slight
5	0.2 gm. egg albumin 1 c.c. horse immune rabbit serum mixed together just before injection	0.2 c.c. horse serum (intravenous) 24 hours after first injection	None
6	1 c.c. horse immune rabbit serum + 2 c.c. NaCl solution	0.2 c.c. horse serum (intravenous) 24 hours after first injection	Very severe; prostrated. Recovery

The usual method of studying the absorption and persistence of antitoxin in the circulation is to inject a guinea-pig with a known amount of serum from the injected person or animal and then find how many M. L. D. of the toxin it can withstand. In order to see whether the action of antitoxin is inhibited by another protein as are anaphylactic antibodies, the next experiment was made. (I am much indebted to Dr. P. G. Heinemann for supplying me with standardized toxin and antitoxin and all the horse serum used in these experiments.)

Dilutions of a diphtheria toxin, the M. L. D. of which was 0.002 c.c. (for a guinea-pig weighing 250 gm.), were made so that each cubic centimeter contained 0.008 c.c. Of this dilution, 1.5 c.c. were measured accurately into each of twelve test glasses. Into four of these were measured 3 c.c. of fresh human

serum, heated to 56 C. for one-half hour, and into another four, were measured 3 c.c. of NaCl solution. The contents of a syringe containing approximately 2,000 units of diphtheria antitoxin were made into dilutions that contained in 1 c.c., 0.08 c.c., 0.04 c.c., 0.02 c.c., and 0.01 c.c. units of antitoxin. Of each of these four dilutions, 1.5 c.c. were measured into the test glasses containing human serum and toxin, or NaCl and toxin. To two other test glasses containing the same amount of toxin were added 1.5 c.c. NaCl solution and 3 c.c. human serum. To the two remaining test glasses with toxin, were added 4.5 c.c. NaCl. From each test glass 4 c.c. were measured into syringes, arranged so that they could be rinsed out, and the contents injected into the peritoneal cavity of guinea-pigs. The results are seen in Table 13.

TABLE 13

EFFECT OF HUMAN SERUM ON ANTITOXIC HORSE SERUM

Guinea-Pigs	Weight in Grams	Treatment	Results
1	361	0.01 unit antitoxin 2 c.c. NaCl solution 0.008 c.c. toxin	Dead in 1 day
2	374	0.01 unit antitoxin 2 c.c. horse serum 0.008 c.c. toxin	Dead in 4 days
3	391	0.02 unit antitoxin 2 c.c. NaCl solution 0.008 c.c. toxin	Dead in 2 days
4	390	0.02 unit antitoxin 2 c.c. horse serum 0.008 c.c. toxin	Dead in 6 days
5	390	0.04 unit antitoxin 2 c.c. NaCl solution 0.008 c.c. toxin	Dead in 7 days
6	402	0.04 unit antitoxin 2 c.c. horse serum 0.008 c.c. toxin	Dead in 10 days
7	418	0.08 unit antitoxin 2 c.c. NaCl solution 0.008 c.c. toxin	Lived
8	420	0.08 unit antitoxin 2 c.c. horse serum 0.008 c.c. toxin	Lived
9	436	2 c.c. horse serum 0.008 c.c. toxin	Dead in 4 days
10	440	2 c.c. horse serum 0.008 c.c. toxin	Dead in 4 days
11	382	0.008 c.c. toxin	Dead in 1 day
12	397	0.008 c.c. toxin	Dead in 1 day

Within the limits of accuracy of the method there was no perceptible effect of human serum on the antitoxic action of horse serum.

SUMMARY OF EXPERIMENTS

The blood or thoracic duct lymph of dogs injected with large amounts of horse serum subcutaneously, even under high pressure, will not sensitize guinea-pigs to horse serum.

Dog serum will inhibit almost completely the active sensitization of guinea-pigs by horse serum mixed with it before injection. Dog serum will also inhibit the active sensitization of guinea-pigs by egg protein mixed with it before injection.

Cat and human sera, and egg protein will inhibit active sensitization by horse serum mixed with them, in no way differently from dog serum.

Horse serum also inhibits active sensitization by egg protein.

Egg protein inhibits active sensitization to horse serum if injected before the sensitizing dose of horse serum.

Horse serum has no effect on the toxigenic action of egg protein in animals sensitized with egg protein, and egg protein has no effect on the toxigenic action of horse serum in animals sensitized with horse serum.

Egg protein inhibits the passive sensitization of guinea-pigs with horse-immune rabbit serum when injected either with or before the sensitizing serum.

Human serum has no effect on the protective action of antitoxic horse serum.

DISCUSSION

In pursuit of an explanation of the phenomena thus described, our experiments have assumed a similarity to those performed by Weil,[4] and ideas expressed by him have been helpful in explaining our results. He has shown that if a guinea-pig is given several injections of comparatively large amounts of normal rabbit or sheep serum, it cannot be passively sensitized to horse serum with horse-immune rabbit serum one to fourteen days after the preliminary treatment. Moreover, guinea-pigs given a sensitizing dose of horse-immune rabbit serum will not be sensitive to horse serum six to nine days later if the sensitizing dose is followed by several injections of normal rabbit or sheep serum. Weil suggests two possible explanations for these results. In the first place, the normal sera, when given before the sensitizing serum, may "saturate" the cell receptors of the body, leaving none available to anchor the immune rabbit serum; and when given

4. Jour. Med. Research, 1913, 28, p. 243.

after the injection of immune serum, they may "displace" the immune bodies. Or, for another possible explanation, the normal sera may give rise to antibodies which prevent the union of antigen and antibody. He did not determine the effect of these proceedings on active sensitization.

Altho Weil is more inclined to accept the latter view as correct, the first one, i. e., "saturation" or "displacement," is more in harmony with our work. The number of cell receptors in the body must be limited and therefore the ability of the cells to take up foreign proteins is limited. Likewise, the capacity of each cell to produce immune bodies or sessile receptors is limited. If every antibody-producing cell in the body were stimulated by an optimal amount of foreign protein, the number of antibodies formed would be the product of the number of antibody-forming cells multiplied by the working capacity of each cell. If the antigen was two chemically distinct proteins in equal amounts, the total number of antibodies formed would be theoretically the same, but the number for each protein would be just one-half because the number of active cells stimulated by each protein is one-half the total number, or, if one cell, having a limited working capacity, can fix more than one protein, the output of each one is reduced one-half.

In our experiments, according to this idea, then, there is inhibition of anaphylaxis for the smaller dose of protein because it was prevented by the protein in excess from uniting in sufficient amount, or was prevented from uniting with a sufficient number of cell receptors, to produce maximal sensitization.

If this is true, the amount of inhibition should depend on the ratio of the amounts of the two proteins injected. All ranges of inhibition should be obtained by varying their proportion. This was put to test experimentally.

Ten guinea-pigs received 0.1 c.c. horse serum intraperitoneally. Together with the horse serum, eight of them received respectively 2 c.c., 1.5 c.c., 1 c.c., 0.5 c.c., 0.25 c.c., 0.1 c.c., 0.01 c.c., and 0.001 c.c. of dog serum. Fourteen days later all received 2 c.c. normal horse serum intraperitoneally. The results are seen in Table 14.

There has been nearly absolute inhibition in the guinea-pigs receiving 2 c.c., 1.5 c.c. and 1 c.c. dog serum. There was some noticeable difference between the one that received only 0.001 c.c. dog serum and the controls.

TABLE 14

THE INHIBITORY EFFECT OF VARYING AMOUNTS OF DOG SERUM ON ACTIVE SENSITIZATION TO HORSE SERUM

Guinea-Pigs	First Injection	Reaction After Second Injection of 2 c.c. Horse Serum Diluted One Half
1	0.1 c.c. horse serum 2 c.c. dog serum	Scratched nose occasionally. Doubtful
2	0.1 c.c. horse serum 1.5 c.c. dog serum	None
3	0.1 c.c. horse serum 1 c.c. dog serum	None
4	0.1 c.c. horse serum .5 c.c. dog serum	Marked; died after 50 hours
5	0.1 c.c. horse serum .25 c.c. dog serum	Severe. Recovery
6	0.1 c.c. horse serum .1 c.c. dog serum	Very severe. Recovery
7	0.1 c.c. horse serum .01 c.c. dog serum	Severe. Recovery
8	0.1 c.c. horse serum .001 c.c. dog serum	Severe; died after about 10 hours
9	0.1 c.c. horse serum	Died in 1 hour
10	0.1 c.c. horse serum	Died in 50 minutes

In the passive anaphylaxis experiments, the immune bodies are "shunted" off from the cells that fix them by the large amount of egg albumin. The two proteins, immune rabbit serum and egg albumin, having an equal chance at the cell receptors, are anchored in proportion to their quantities. The "shunting off," as would be expected, occurs if the egg albumin is injected before, or at the same time as, the injection of the immune rabbit serum. On the other hand, antitoxin does not require binding to the body cells to afford protection. Probably only the antitoxin free in the circulation can be efficient. Hence, from our thesis, it would be expected, as was found, that other heterologous proteins, in whatever amounts, would not inhibit the combining of the toxin with its specific antitoxin.

The fact that the inhibition of passive anaphylaxis occurs if the egg albumin is injected with the immune serum is serious evidence against the idea that antibodies are formed which prevent anaphylactic intoxication, because it would be necessary that these antibodies be formed in twenty-four hours, and it cannot be due to "displacement" because the immune bodies are presumably not yet "placed."

It is admitted that a serious objection to the acceptance of this explanation is that it is against our ideas of specificity. It suggests that two proteins may be anchored by the same receptors. This objection may not be serious. If an antigen can combine only with a specific receptor, it means that the body cells must contain the unbelievable number of receptors that would be required to bind every protein that occurs in nature, and in addition, a large number of modifications of these proteins by various changes produced in the "constitutive Gruppierung." Non-specific antibodies are known to occur normally in the body. Normal human serum not only inhibits proteolysis by homologous enzymes, but also, and even more so, by enzymes of the pig and perhaps other animals.[5]

In these experiments may lie the explanation of the observation made by Wells[2] that sensitization could not be obtained with as small an amount of whole egg-white as of pure, crystallized egg albumin. With whole egg-white the antibody formation is divided between four chemically and biologically distinguishable antigens.

A number of investigators have shown that antigen rests exist in the circulation of immune animals, by showing that the serum of these animals will actively sensitize another one to the antigen. Gay and Southard[6] showed that the serum of a guinea-pig immune to horse serum will sensitize another guinea-pig within fifteen days to horse serum. Hintze[7] gave rabbits a single relatively large dose of horse serum or egg-white, bled them at regular intervals, and injected the serum into guinea-pigs. The guinea-pigs were sensitized to horse serum but not markedly so. The same experiment was made by Jonesco-Mihaiesti,[8] except that still larger doses of antigen were given the rabbits in repeated injections. The same positive results were obtained. The method of Gay and Southard determined more accurately the true amount of antigen rest, because the immune serum was injected into a homologous animal and the inhibitory action of the heterologous serum was not obtained as in the experiments of Hintze and Jonesco-Mihaiesti. That the latter obtained active sensitization at all with the immune serum is probably due to the fact that the rabbits were immunized with relatively large doses of antigen, and enough was retained in the circulation to overcome the inhibition of the rabbit serum.

5. Von Eisler, Ber. d. Wien. Akad., 1905, 104, p. 119.
6. Jour. Med. Research, 1907, 16, p. 143.
7. Ztschr. f. Immunitätsforsch., 1910, 6, p. 113.
8. Compt. rend. Soc., de biol., 1911, 70, p. 104.

A number of investigators have sensitized animals with two or more proteins in the study of the specificity of antianaphylaxis. As controls were not made in these experiments by sensitizing animals with the same amount of one protein alone, the mutual inhibition of anaphylactic sensitization to the several proteins was apparently not observed. But our attention has been called to the work of Benjamin and Witzinger[9] who have made experiments exactly similar to some of ours in active sensitization. They found that a large dose of horse serum would inhibit the sensitization of a guinea-pig to beef serum given at the same time or twenty-four hours later. They also found that a preliminary injection of horse serum would inhibit the formation of hemolysins by rabbits for sheep corpuscles and that a large dose of beef serum would inhibit the formation by rabbits of precipitins for horse serum, thus giving further support to the idea of saturation of non-specific receptors as the cause of inhibition.

GENERAL SUMMARY

An amount of protein that will produce a marked anaphylactic sensitization when injected alone into a guinea-pig, will fail to do so if injected together with, or twenty-four hours after, a much larger amount of another protein.

The serum from a rabbit immune to horse serum, which will markedly sensitize a guinea-pig to horse serum, will fail to do so if injected with, or twenty-four hours after, a large dose of another protein.

These results may be explained by the conception that the number of receptors in the body that can unite with a foreign protein is limited. The inhibiting protein, if present in large amount, combines with all, or almost all, of these receptors. Hence, another protein injected with it, or after it, is prevented from being combined in sufficient amount to stimulate the active production of antibodies. And when a large amount of protein is injected with or after a sensitizing dose of immune serum, the combination of the latter with the cell receptors, which is necessary for passive sensitization, is prevented in the same way.

9. Ztschr. f. Kinderheilk., 1911, 3, p. 73.